Oral HPV Shedding and Warts after Starting Antiretroviral Therapy: ACTG Protocol A5272

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BACKGROUND

Both oncogenic human papillomavirus (HPV) subtypes associated with the pathogenesis of oropharyngeal cancer and non-oncogenic subtypes associated with the development of oral warts are found in the oral cavity. Studies exploring the presence of HPV DNA (a marker of active HPV replication) in the mouth have reported that 14% to 35% of HIV-infected adults shed oral HPV DNA, a proportion substantially higher than reported in non-HIV-infected populations, and that oncogenic HPV subtypes are more prevalent in HIV-positive than HIV-negative men who have sex with men. There are also recent reports of an increasing incidence of HPV-related oropharyngeal malignancies among the US population living with HIV infection, despite the widespread availability of effective, combination antiretroviral therapy (ART) for almost two decades. However, the effect of ART and associated immune reconstitution on HPV replication in the oropharynx has not previously been prospectively examined.

Retrospective studies have reported that the use and increased duration of ART were associated with the detection of HPV DNA in oral secretions and that oral HPV DNA has been detected more frequently in HIV-infected patients who were virologically suppressed than those not suppressed on ART. Studies have also suggested that the prevalence of HPV-associated oral warts is increased in HIV-infected patients after initiating ART. These findings are counterintuitive since the degree of immune reconstitution that results from virologically effective, combination ART controls other AIDS-associated, opportunistic infections caused by DNA viruses such as herpes simplex virus, cytomegalovirus, Epstein-Barr virus, and JC virus.

These retrospective findings led us to hypothesize that active HPV replication in the mouth and oropharynx is highly prevalent in HIV-infected patients and persists after ART initiation. If true, HPV shedding and the potential for development of HPV-related oral warts and carcinomas might be expected to increase among HIV-infected individuals on ART.

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OBJECTIVES

In ACTG protocol A5272, we sought to explore prospectively:

1) the changes that occur in oral HPV shedding among treatment-naïve HIV-positive adults during the first 24 weeks after initiating ART
2) the prevalence of oral warts prior to ART initiation and incidence over 48 weeks follow-up
3) Oral HPV shedding in relation to immune reconstitution as measured by change in CD4 cell count following ART, and in relation to control of HIV RNA viral load replication

METHODS

STUDY DESIGN AND POPULATION

- A5272 was a prospective, observational study of HIV-1 infected ART-naïve adults (18 years or older) initiating ART as part an ACTG or industry-sponsored ART treatment trial
- Participants screened for A5272 when they were enrolling in an ART treatment trial at participating ACTG Clinical Research Sites (CRS) before initiation of ART
- Study duration: 48 weeks; Participants seen for 5 visits
- All participants provided written informed consent. The protocol was approved by the institutional review boards of all participating sites

VARIABLES AND MEASURES

- Participants seen
  - for two visits scheduled within one week prior to ART initiation (study pre-entry and entry),
  - then during a 12-18-week time-interval following ART initiation,
  - and at 24 and 48 weeks after ART initiation
- Whole saliva and throat-wash specimens collected at each of the four time points (study pre-entry, study entry, weeks 12-18, week 24)
- An oral soft tissue examination was performed at entry and weeks 12-18, 24, and 48
- CD4+ T cell count and plasma HIV-1 RNA were obtained at entry and weeks 4, 12-18, and 24 (or at discontinuation if a participant exited the study prematurely)
- A detailed medical history was obtained at study entry
- Real time PCR reactions were performed using Roche Lightcycler TaqMan master mix. Each reaction consisted of 1X Roche Lightcycler TaqMan master mix, HPV specific primer pairs and fluorescently-tagged probes for types 6, 11, 16 and 18 in a total reaction volume of 10 mL. Real time PCR reactions that target the E1 region of HPV were performed using Roche Lightcycler Sybr Green master mix, and sequencing of PCR products was performed as previously described

METHODS

STATISTICAL ANALYSES

We computed:

- Standard summary statistics to describe sample
- Prevalence of oral warts at study entry, and incidence over the 48 weeks following ART initiation
- Baseline prevalence of oral HPV infection defined as having HPV DNA of any type present in at least one of the two throat wash specimens collected at the study pre-entry or entry visits*
- Proportion of participants who developed oral HPV infection at one of the two follow-up visits (after initiating ART) among those who did not exhibit oral HPV shedding at study pre-entry or entry*
- Oral type-specific HPV infection at baseline and at 12-24 week follow-up
- Proportion of persisters, clearers, and acquirers for oral oncogenic HPV subtypes versus non-oncogenic subtypes, and by gender
- Median change in absolute CD4+ T cell count and HIV viral load from study entry to three follow-up time points: weeks 4,12-18, and 24

* We collected two throat wash pre- and post-initiation of ART to increase accuracy of oral HPV infection prevalence and incidence estimates. Analyses included only participants who had available specimens for the 4 time points

RESULTS

Figure 1. Frequency of each HPV subtype at baseline (pre-entry and entry)

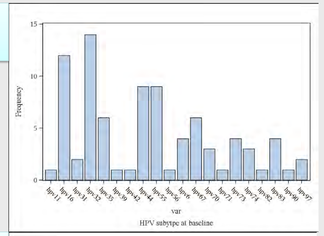
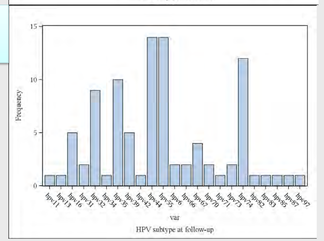


Figure 2. Frequency of each HPV subtype at follow-up (12-18 weeks and 24 weeks)



RESULTS

Table 1. Characteristics of participants (N = 388)* in ACTG 5272 enrolled at 39 Clinical Research Sites from January 2, 2010 to June 4, 2012

Characteristics	N (%)
Sex	
Women	75 (19)
Men	313 (81)
Race / Ethnicity	
White, non-Hispanic	139 (36)
Black, non-Hispanic	152 (39)
Hispanic	87 (22)
Other	10 (3)
Intravenous Injection Drug use	
Never	365 (94)
Previously	23 (6)
	Median (Q1, Q3)
Age at study entry	38 (29–47)
CD4+ T cell count (cells/mm^3)	
• at study entry (N = 385)	328 (174–455)
• 4 weeks after initiating ART (N = 359)	402 (249–559)
• 12-18 weeks after initiating ART (N = 388)	447 (290–592)
• 24 weeks after initiating ART (N = 387)	483 (311–647)
Plasma HIV-1 RNA (log_{10} copies/mL)	
• at study entry (N = 387)	4·68 (4·20–5·22)
• 4 weeks on ART (N = 379)	2·40 (1·81–2·89)
• 12-18 weeks on ART (N = 388)	1·59 (1.59–1.77)
• 24 weeks on ART (N = 387)	1·59 (1·59–1·59)

* Participants who had evaluable throat wash HPV DNA results from both time points before initiating ART and again after 12-18 and 24 weeks of ART

Table 2. Prevalence of Oral HPV DNA shedding among participants in A 5272 at baseline (measured as a positive throat wash for HPV DNA at pre-entry or entry) and at follow-up (measured as a positive result at weeks 12-18 or week 24)

	Total n/N* (%)	Men n/N* (%)	Women n/N* (%)	*P*-value†
Prevalence of any oral HPV subtype at pre-entry or entry	70/388 (18%) [14%–22%]	62/313 (20%) [16%–25%]	8/75 (11%) [5%–20%]	0·06
Prevalence of any oral oncogenic HPV subtype at pre-entry or entry	18/388 (5%) [3%–7%]	14/313 (4%) [2%–7%]	4/75 (5%) [1%–13%]	0·75
Prevalence of any oral HPV subtype at weeks 12-18 or week 24	93/388 (24%) [20%–29%]	71/313 (23%) [18%–28%]	22/75 (29%) [19%–41%]	0·23
Prevalence of any oral oncogenic HPV subtype at weeks 12-18 or week 24	21/388 (5%) [3%–8%]	14/313 (4%) [2%–7%]	7/75 (9%) [4%–18%]	0·10

•The denominator are participants who had evaluable throat wash HPV DNA results at both visits before and after initiating ART
† Chi-square test comparing HPV prevalence between men and women

RESULTS

Table 3. Comparison of absolute CD4+ T cell change, and HIV RNA load, over time among those who did not develop oral HPV shedding, and among those who did

CD4+ T cell count change after ART initiation		Participants with no oral HPV shedding over 24 weeks	Participants who developed HPV oral shedding within 24 weeks after ART initiation	*P*-value*
at week 4	Median [Q1–Q3]	(n = 245) 79 [20–132]	(n = 47) 125 [54–213]	0.003
at weeks 12-18	Median [Q1–Q3]	(n = 267) 116 [53–195]	(n = 48) 163 [77–274]	0.04
at week 24	Median [Q1–Q3]	(n = 267) 144 [71–245]	(n = 267) 190 [94–292]	0.08
log_{10} HIV RNA Load change after ART initiation				
at week 4	Median [Q1–Q3]	(n = 264) 2 [2–3]	(n = 48) 2 [2–3]	0.34
at weeks 12-18	Median [Q1–Q3]	(n = 270) 2 [2–2]	(n = 48) 2 [2–2]	0.73
at week 24	Median [Q1–Q3]	(n = 270) 2 [2–2]	(n = 48) 2 [2–2]	0.79

* Mann-Whitney test

Detection of Oral Warts

- At entry, 3% (95%CI: 2%–5%) of participants were found to have oral warts.
- After 48 weeks of follow-up, 2.5% (95%CI: 1%–4%) of subjects who did not have oral warts at entry developed oral warts.

CONCLUSIONS

- No reduction in oral HPV DNA prevalence after 12 to 24 weeks of ART despite a high rate of HIV virologic suppression and robust immune reconstitution as indicated by absolute CD4+ T cell count increase
- The prevalence of any oncogenic oral HPV subtype was 5% both before and after 12-24 weeks of ART
- Among those who were HPV DNA negative before initiating ART, 25% had evidence of a new HPV infection during the first six months of ART
- The finding that HPV replication occurred more frequently among those who had the largest increases in circulating absolute CD4+ T cell counts does suggest that activation of latent HPV infection may be an epiphenomenon of immune reconstitution in HIV patients
- Given the increased incidence of HPV-associated oropharyngeal cancer that has been reported in the last two decades, our findings are concerning.
- Studies that can define how HIV, despite ART, permits unrestricted HPV replication in the oral cavity are needed to provide the scientific underpinning for preventive strategies other than HPV vaccination in young men